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ELECTRODES FOR FUNCTIONAL NEUROMUSCULAR STIMULATION

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Section B: Electrode Design and Fabrication

B.3 Electrode Materials

Abstract

A series of in vivo tests in rats has been performed to test the biological response to alternative silicone rubber and fluoropolymer materials used in the manufacture of electrodes. Preliminary histological evaluation of some of the tissue samples has begun and is briefly described here. Histological processing of the remaining tissue samples is ongoing.

Background

Silicone rubber nerve cuff electrodes were implanted on the sciatic nerves of adult rats. Segments of fluoropolymer insulated wire were placed subcutaneously on the backs of these same animals. Original and replacement materials were used in the manufacture of these implants. Two and 4 weeks after implantation, the animals were killed by aortic perfusion and the tissue was fixed.

Tissue Sample Preparation

After fixation, the implants and surrounding tissue were excised from the animal carcass and placed in individual vials of sodium cacodylate buffer. Super VHS video recordings were made during dissections of each implant sample in preparation for histological processing.

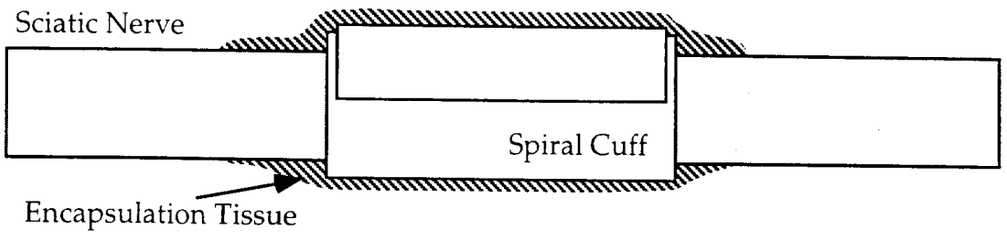
Nerve Cuffs

The implanted nerve cuffs and lengths of sciatic nerve were removed from the animal carcass with special care as to not destroy the surrounding encapsulation and to not displace the cuff from the nerve. Additionally, efforts were made to include a minimum of 1 cm length of nerve both proximal and distal to the cuff edges in the excised tissue sample. Excised nerve cuff samples are depicted in Figure 1.

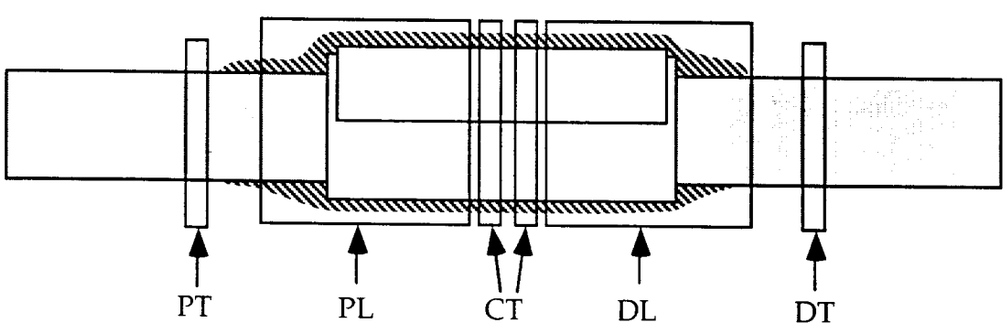
These studies were intended to investigate the tissue response to the implants, including both the inflammatory response to the materials as well as the neural response to the surgical intervention and the presence of the cuff. Therefore, from the tissue samples, both nerve and encapsulation tissue will be histologically processed and stained. Samples for encapsulation tissue response will be embedded in paraffin and stained with both Hemotoxylin & Eosin and Trichrome; samples for neural tissue response will be embedded in plastic and stained with Methylene Blue.

With the cuff still in place on the nerve, straight razor blades were used to cut through the outer encapsulation, the silicone rubber wraps of the cuff, the inner encapsulation, and the nerve itself, as shown in Figure 1. Transverse nerve sections were taken at proximal (~3-4 mm from cuff edge), distal (~3-4 mm from cuff edge) and mid-cuff levels for staining with

Methylene Blue and were labeled PT, DT, and CT respectively. An additional transverse section was taken at the cuff level for H&E and Trichrome staining. Longitudinal sections were taken at both the proximal and distal portions of the cuff, labeled PL and DL respectively. These sections were then cut in half longitudinally; one half to be paraffin embedded and one half to be plastic embedded. After preparing the samples from each excised nerve, the pieces of silicone rubber from the cuff were removed from each piece with fine forceps, leaving the outer encapsulation in place when possible. All steps were recorded on videotape, rough drawings were made of each sample, and the samples were placed in individual labeled vials of buffer solution.



Sections taken from each nerve:



PT: proximal transverse (plastic embed)
PL: proximal longitudinal (plastic & paraffin embed)
CT: center transverse (plastic & paraffin embed)
DL: distal longitudinal (plastic & paraffin embed)
DT: distal transverse (plastic embed)

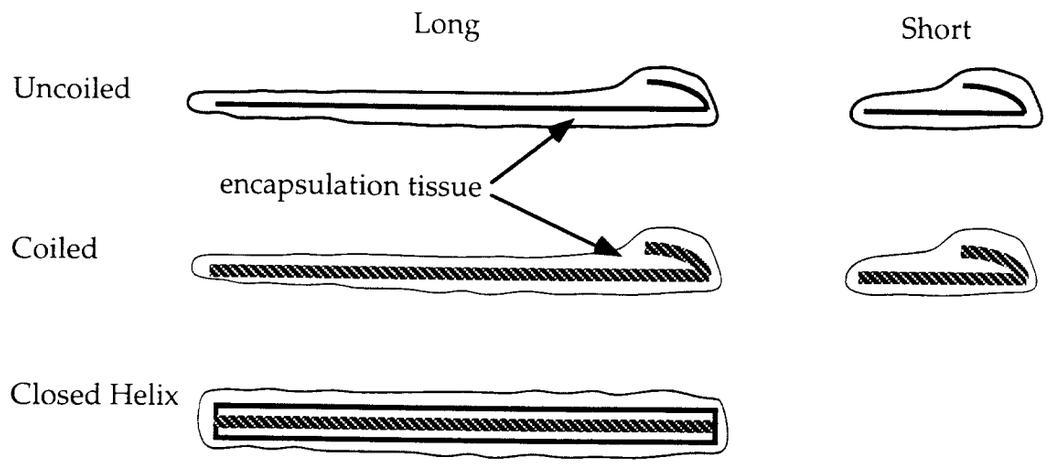
Figure 1: Top: Schematic drawing of excised nerve cuffs, including encapsulation tissue, proximal and distal lengths of the sciatic nerve, and the implant itself. In the figure, the encapsulation tissue is depicted behind the nerve and cuff, although it covered all sides of the cuff. Bottom: Schematic depiction of where samples were taken from each excised nerve.

Wire Samples

Insulated wires were implanted subcutaneously along the back of the rats. The samples were of two lengths: long (7cm) and short (1 cm); were of two configurations: coiled and uncoiled; and were of two insulating materials: PFA and FEP fluoropolymer. Additional samples of closed helix lead (coiled wire inside silicone rubber tubing) were also implanted.

Encapsulated wires were removed by carefully peeling back the skin and exposing the subcutaneous space and underlying tissue. Under direct vision and using blades and fine scissors, the wire samples and surrounding encapsulation tissue were excised from the rat carcass. The encapsulation tissue was prepared for paraffin embedding and histological staining with H&E and Trichrome. Longitudinal samples 1-2 cm in length were taken from each implant. For the long wire implants, a transverse section was also taken. These are depicted in the following figure. Razor blades were used to cut through the encapsulation tissue and the implanted wire, and then the wire was removed from the tissue with fine forceps. All steps were recorded on videotape, rough drawings were made of each sample, and the samples were placed in individual labeled vials of buffer solution.

Wire Implants:



Sections taken from each wire implant:

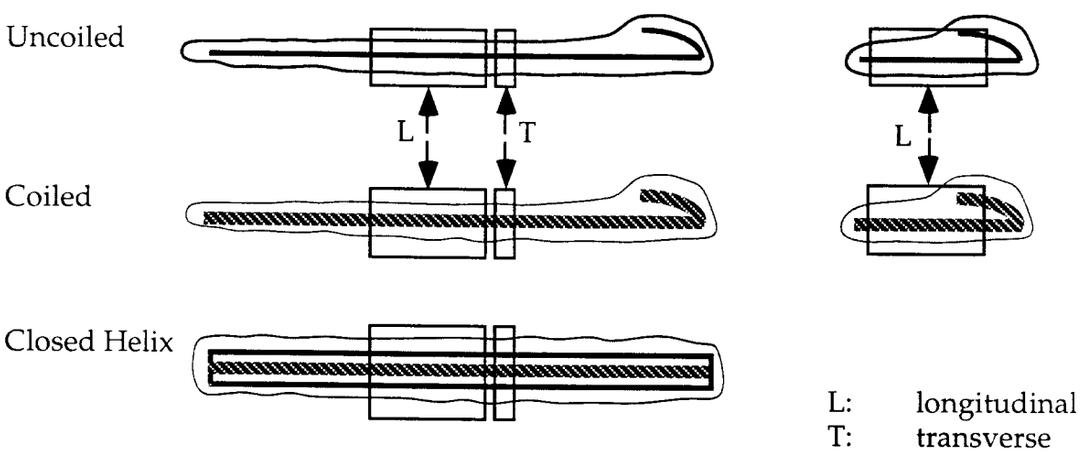


Figure 2: Top: Schematic drawing of excised wire implants, including encapsulation tissue and the implant itself. In the figure, the encapsulation tissue is depicted behind the wire, although it covered all sides of the implant. Bottom: Schematic depiction of where samples were taken from each excised implant.

Histological Evaluation

Paraffin embedding, microtome sectioning, and H&E and Trichrome staining was performed at the Pathology Lab of University Hospitals of Cleveland. Stained slides have been received for the implants from the first 12 rats in the study; the remaining slides are in the process of being prepared. Plastic embedding, microtome sectioning, and Methylene Blue staining of sections is being performed at the Electron Microscopy Lab of CWRU Medical School; the slides are in the process of being prepared.

Preliminary evaluation of the encapsulation tissue has begun. To reduce bias in the evaluation of the cellular response to the materials, the slides are being examined with only the 3-4 digit sample code number as reference. No comparisons between the cellular response to specific implant materials will be made in this report, as the evaluation is not complete.

Nerve Cuffs

Examples of PL, CT, and DL nerve cuff sections are presented in the following figures (Figures 3-5). In these samples, the capsules were primarily characterized by multiple layers of elongated fibroblasts and associated developing collagen. The orientation of the fibroblasts was noted to be consistent with the contours of the implant. Inflammatory cells, including rounded macrophages and fibroblasts, occasional foreign body giant cells and polymorphonuclear cells, were primarily limited to the first few cell layers at the capsule edge nearest the implant. Often a variable level of inflammatory response, both in number and kind of cells, was observed within the same sample. Additionally, a general increase in cellularity and inflammatory response was found in distal sections as compared to proximal sections. Most tissue sections were well vascularized, and in some samples red blood cells were observed still within vessels and/or surrounding tissue. A robust collagen layer was found surrounding the nerve fascicles, and was both thicker and more organized than the collagen found within the capsule. Possible polymer fragments were observed in several sections, supported by the fragments exhibiting birefringence under polarized light and appearing to be surrounded by macrophages and/or foreign body giant cells.

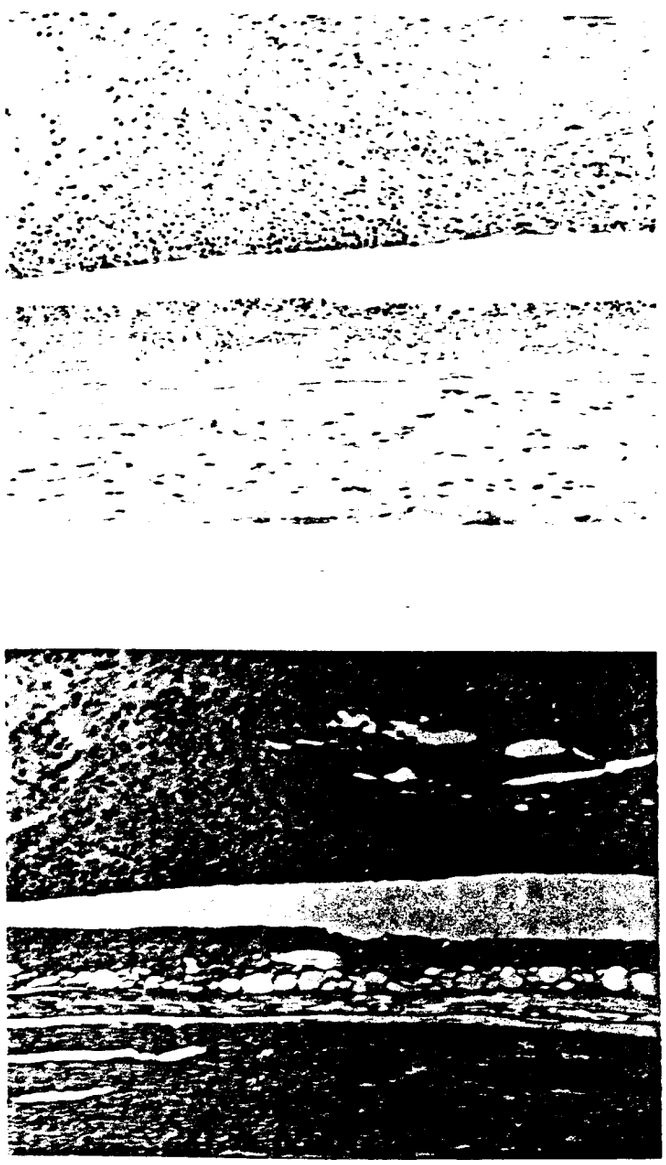


Figure 3: Photomicrographs of PL (proximal longitudinal) section from cuff implant under normal (top) and polarized (bottom) light. One wrap of the cuff had lain in the blank space in the middle of the figures. Inflammatory cells are found on either side, with underlying elongated fibroblast and collagen layers. In the lower half of the capsule, a layer of fat cells are present, followed by a layer of collagen surrounding the nervous tissue, which is shown at the bottom third of the figure. Under the polarized light, a possible polymer fragment is seen glowing, as is the well-organized collagen surrounding the nerve. (R9 #122PL, H&E, 95x)

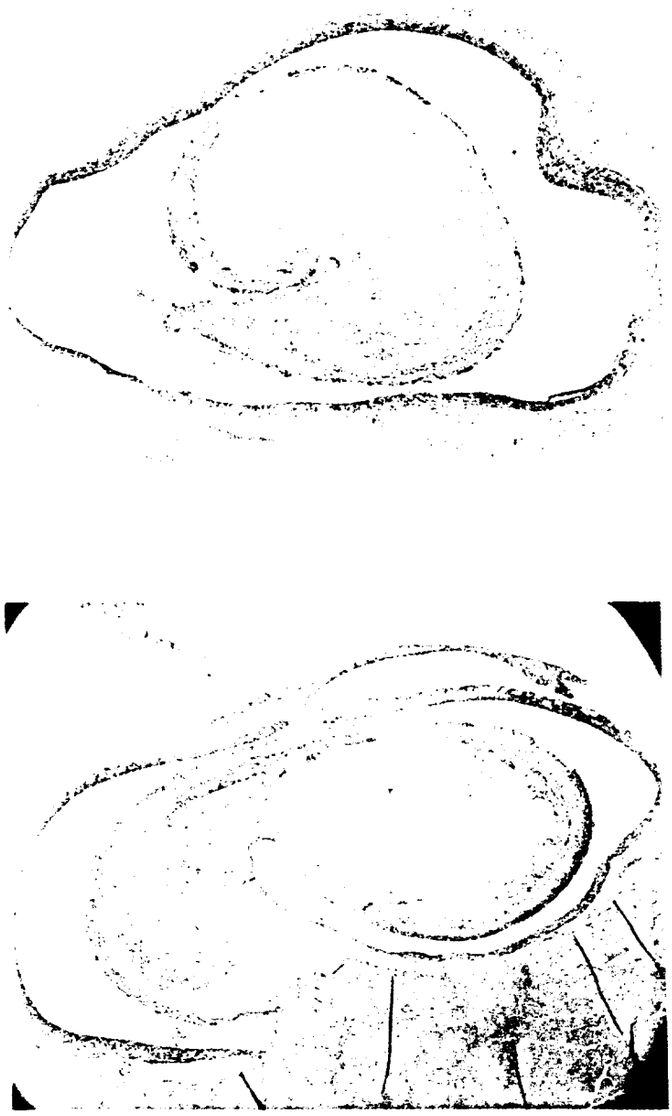


Figure 4: Photomicrographs of CT (center transverse) sections from cuff implants. Top: Inner encapsulation and nerve are separate from outer encapsulation piece (orientation is not accurate). Varying thickness of both the outer and inner capsule is noted. (R12 #212CT, H&E, 37.5x) Bottom: Nerve, inner and outer capsule are continuous and supported by muscle tissue shown in lower right of figure. Varying thickness of the capsule is noted, as is the rich vascularity within both the inner and outer capsules. (R10 #113CT, H&E, 25x).

Figure 5: Photomicrograph of DL (distal longitudinal) section from cuff implant. The capsule is in the top portion of the figure, with the distal end shown at the left edge. Below the capsule is a layer of collagen, followed by the nervous tissue. (R1 #126DL, H&E, 37.5x)

Uncoiled Wires

Examples of both L and T sections from uncoiled wire implants are shown in the following photomicrographs (Figure 6). The capsules from these uncoiled wires were also dominated by multiple layers of elongated fibroblasts and associated developing collagen. Inflammatory cells, such as macrophages, rounded fibroblasts, and occasional foreign body giant cells, were limited to the first few cell layers of the capsule. However, the thickness of this inflammatory band of cells was found to vary. Additionally, red blood cells were noted both within vessels and within the tissue.

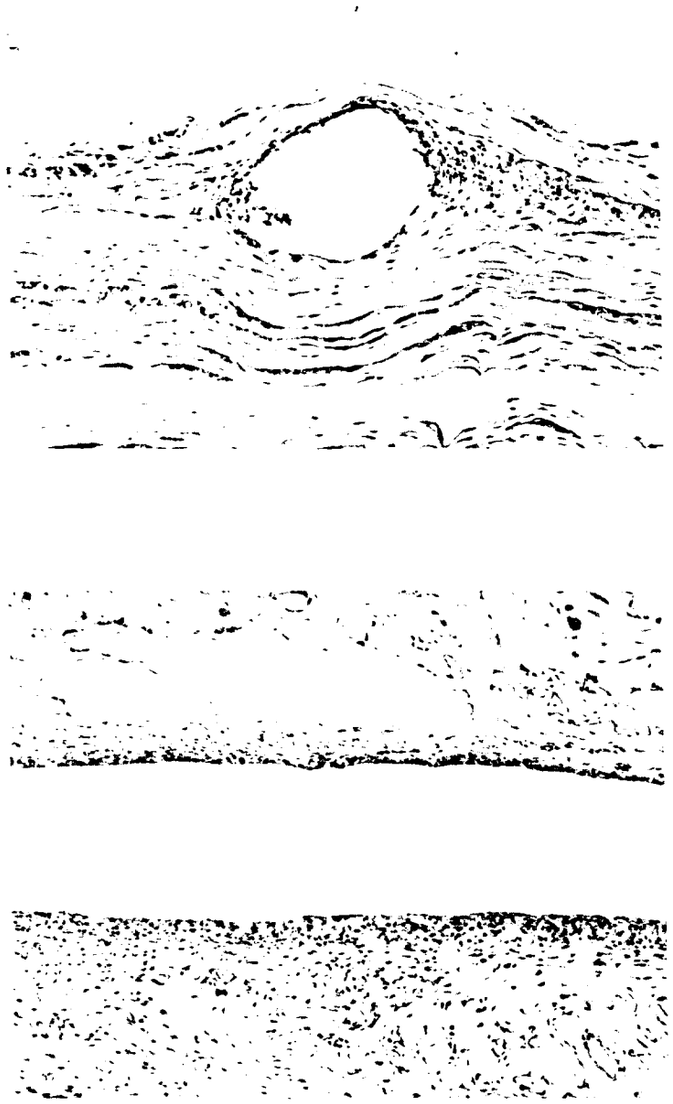


Figure 6: Photomicrographs of transverse (top) and longitudinal (bottom) sections from uncoiled wire implants. Top: Transverse section. A few inflammatory cells are present, although the capsule is predominantly characterized by elongated fibroblasts. (R8 #0103, H&E, 95x). Bottom: Longitudinal section. Slightly increased inflammation is seen in the lower capsule as is a rich vascular bed. (R3 #0113, H&E, 95x).

Coiled Wires

Examples of both L and T sections from coiled wire implants are shown in Figure 7. Again, the dominant feature of the capsules from these implants was multiple layers of elongated fibroblasts and developing collagen, with the fibroblasts being oriented along the contours of the implant and around each coil. The elongated fibroblast layers and associated collagen was especially evident along the outer edge of the coils. Increased inflammation was noted in the central areas of the coils, particularly in the tissue between individual coils, with the presence of frequent macrophages, foreign body giant cells and polymorphonuclear cells. The capsules were generally well vascularized, but often contained large numbers of red bloods within the tissue, which would appear to indicate localized hemorrhage.

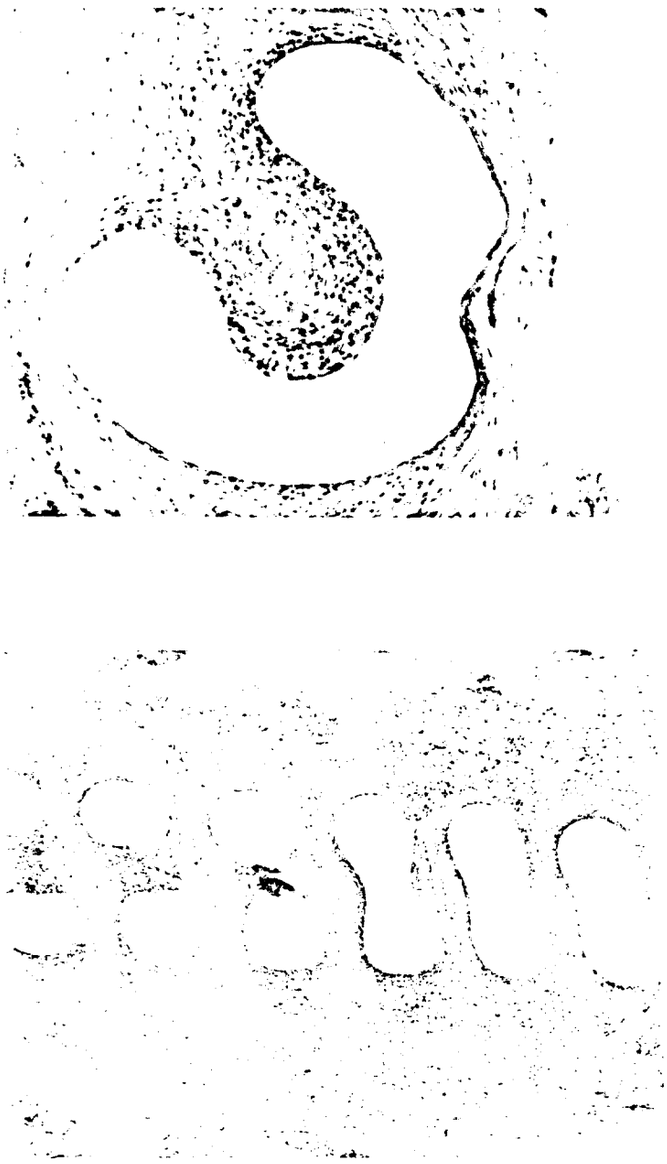


Figure 7: Photomicrographs of transverse (top) and longitudinal (bottom) sections from coiled wire implants. Top: Transverse section. The outer capsule is comprised of elongated fibroblasts and occasional inflammatory cells, while the tissue between the coils (center) has many inflammatory cells and vessels. (R5 #1004, H&E, 95x). Bottom: Longitudinal section. Capsule markedly thickens in the center of the coils, particularly in relation to regions of the outer edge that contain only a few elongated fibroblast layers. (R5 #0001, H&E, 37.5x).

Closed Helix Lead

Examples of both L and T sections from closed helix lead implants are shown in Figure 8. Capsules from these implants contained multiple layers of elongated fibroblasts and developing collagen. Inflammatory cells, including macrophages, rounded fibroblasts, and polymorphonuclear cells, were primarily limited to the first few cell layers of the capsule. In many of the samples, the lead developed a loop in the middle and on gross observation, these loop regions appeared to be hemorrhaged. Histologic samples from these regions show many red blood cells, consistent with the hemorrhaged appearance.

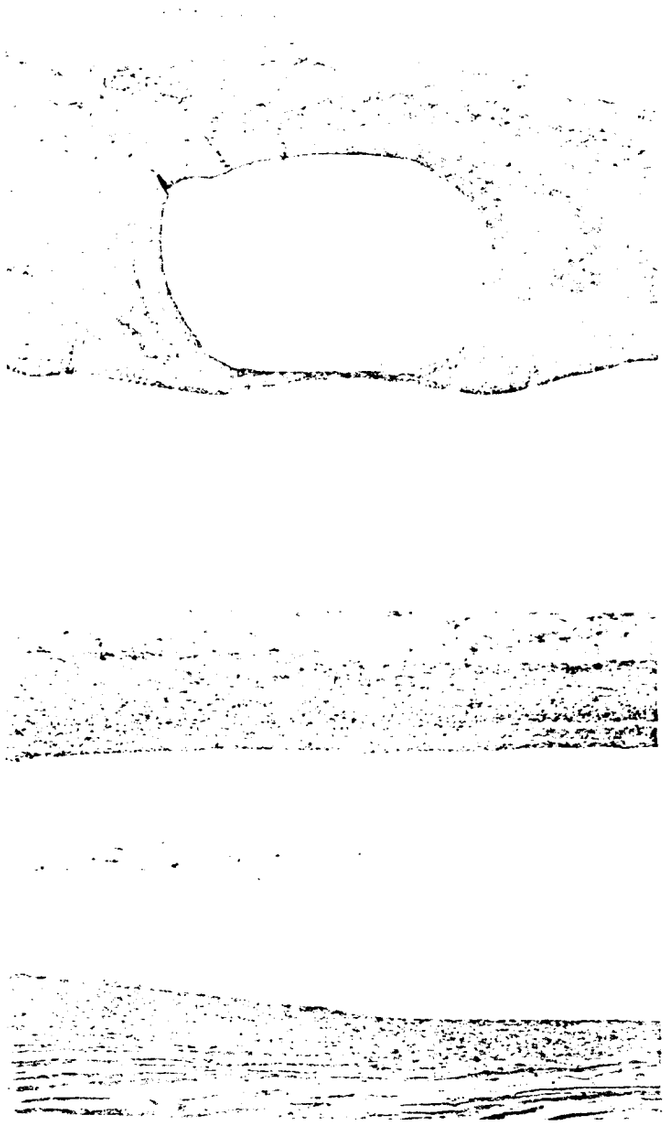


Figure 8: Photomicrographs of transverse (top) and longitudinal (bottom) sections from closed helix implants. Top: Transverse section. The capsule is predominantly characterized by elongated fibroblasts, and is surrounded by many blood vessels. (R3 #2203, H&E, 37.5x). Bottom: Longitudinal section. Capsule is shown to have varying thickness, but is predominantly elongated fibroblasts. (R12 #2205, H&E, 37.5x).

Future Work

The histological evaluation will continue as the remaining slides should be received shortly. After completion of the histological evaluation, observations will be correlated to specific implant material and implant duration. The effects of implant material, configuration, and duration will be evaluated by any trends in number or presence of specific inflammatory cells, incidence of hemorrhage, and development of surrounding collagen. Similar analysis will be performed on the neural tissue.

Section C: Assessment of Electrode Performance

C.4 Surgical Trauma During Implantation of Multiple Contact Cuff Electrodes

Abstract

Short term chronic studies involving monopolar cuff electrode implants were carried out in this quarter. The experiments were designed to investigate the effects on the nerve due to the surgical procedure and those due to the short-term presence of the cuff and leads. A standard implant was performed on one limb of each animal, while on the sham limb, the cuff was removed after being placed on the nerve. After 10 days, the animals were killed by aortic perfusion, and the neural tissue and surrounding encapsulation were excised for future histological processing.

Background

Self-sizing spiral nerve cuff electrodes have been used in numerous studies, both acute and chronic, performed in our laboratory. The acute experiments have typically been investigations of the selective recruitment that can be achieved through the use of the cuff. Any histological processing of nerve tissue from those acute experiments was intended to establish nerve fascicle position relative to electrode contact position. The chronic experiments that have been performed investigated both the long-term safety and the recruitment stability of the cuff. From those studies, long-term (3-6 month) tissue response to the nerve cuffs has been evaluated. However, the short-term effects of the implant procedure itself, as well as the short-term effects of the cuff and lead cable on the nerve, has not previously been studied.

Histological analysis of nerve tissue from the chronic implants performed in this contract period revealed some morphological changes in the nerves. These changes appeared to be resolving and were speculated to be due to the surgical procedure. However, the implants in that study contained a bulky, 12-wire uncoiled lead cable that may have produced tethering and mechanical irritation of the nerve trunk, contributing to the morphological changes within the nerve. These observations require that additional studies be performed and issues regarding the degree of surgical trauma associated with spiral nerve cuff implant be resolved. Because the lead cable may have played a role in the observed changes, lead cable modifications should also be considered.

In previous quarters (QPRs #9, 10), we have reported our efforts involving the 4-contact monopolar cuff as a replacement to the 12-contact tripolar cuff. Those studies were initiated because of our concerns about the clinical feasibility and safety of a 12-wire lead cable. The results have indicated that the monopolar design has similar selective recruitment capabilities as the tripolar cuff. In addition to ease of manufacture and

implant, the reduced number of contacts results in a smaller lead cable. The reduced number of lead wires makes helical coiling of the lead cable feasible, unlike the case for 12 wires. Helical coiling of the lead cable provides a substantial mechanism for stress relief along the cable, lowering the risk of stress transfer from the cable to the nerve trunk. As the monopolar design appears to be promising for future clinical implementation, 4-lead electrodes were used in the studies described here.

The experiments performed in this quarter involved sham and actual implants of spiral nerve cuff electrodes in 4 adult felines. The experiments were terminated after 10 days to allow for histological analysis of the *short-term* neural response. Each animal in the study included a control (right side) and an experimental (left side) nerve. The surgical control was achieved by performing the full implant procedure, with the exception that once the cuff was in position, it was then carefully removed. On the experimental side, the full implant procedure was performed, and the cuff was left in place. Nerves from the surgical control side will provide an indication of the trauma inflicted on the nerve due solely to the implant procedure. Any additional trauma found in nerves from the experimental side can then be attributed to the presence of the cuff and lead cable.

Methods

Cuff Preparation

Standard manufacturing procedures were followed in the preparation of the implants. Each cuff was made to a thickness of 125 µm using one of our replacement silicone rubber sheeting batches (MED2-6400, NuSil) that is being investigated in the rat studies described in QPRs #8-10. No platinum contacts were mounted on the lead wires or embedded within the silicone rubber cuff, as no stimulation was to be performed during these implants. Small caliber wires, 1x7x0.0008" (standard size is 1x7x.00135"), were used in the lead, which consisted of 4 wires helixed together into a single cable. Standard cleaning procedures were used to eliminate any dirt or grease from the cuffs. After packaging, the cuffs were sterilized by exposure to ethylene oxide and were allowed to de-gas a full week before being used for implant.

Surgical Procedure

The surgical procedure was performed using standard aseptic techniques, following the same protocols typically used in cuff implant. Adult felines were used in these studies. Each animal was first sedated with Rompun (2.0 mg/kg, SC) and then anesthetized with a Halothane/Oxygen mixture. An IV line was established in the proximal forelimb for fluid maintenance and the animal was intubated to maintain a constant airway. A 100 mg pre-surgical dosage of an antibiotic (Oxacillin Sodium) was administered IM. The surgical area was shaved and remaining hair stubble was removed using a depilatory cream. The incision site was scrubbed with a surgical prep solution, and the animal was covered with sterile drapes.

The electrode was placed in a saline and Kefzol® bath prior to implant. The upper hind limb was surgically opened to expose the sciatic nerve just proximal to the popliteal fossa. A 2 cm length of nerve was isolated from surrounding connective tissue using glass hooks, and the electrode was carefully placed around the nerve, forming a snug, uniform fit. The lead wires were positioned to exit distally, and were then looped to extend proximally towards the hip. An additional loop was formed as the lead exited the muscle plane and then the lead was tunneled subcutaneously to the hip joint. A final loop was made in the lead at the hip joint and the surgical site was sutured closed.

The right side, the surgical control, was performed first and then the left side, the experimental, was actually implanted using the same cuff as had been used on the right. Both procedures followed those described above, except that on the right side, the cuff and lead wires are removed and returned to the saline and Kefzol® bath just before closing the surgical site. Once the incisions were closed, a 100 mg post-surgical dosage of Oxacillin Sodium was administered IM. The animals were taken off the Halothane/Oxygen mixture and monitored for post-anesthesia recovery.

Post-Surgical Observation

All animals were observed post-surgically for signs of pain, as evaluated through movement and usage of the hindlimbs. Additionally, observations were made over the course of the implant period of the animals' behavior, gait pattern, presence or absence of hock drop, presence or absence of knuckling of rear paws, and general attitude and activity level. Neurological examinations, including the checking of paw withdrawal to light pinching and toe-tapping during gait to observe limb corrections (placement reactions), were performed multiple times over the 10 day period.

Perfusion Procedure

At the end of the 10 day incubation period, each animal was killed by aortic perfusion. The animal was anesthetized with a combination of Ketamine (30 mg/kg, IM), Atropine (0.044 mg/kg, IM) and Sodium Pentobarbital (0.2 cc bolus injections, IV). The animal was intubated, and rectal temperature was maintained at 39° C with a thermostatically controlled heating pad. The animal was transferred to a respirator, the chest was opened, and the pericardium cut away from the heart. An incision was made in the right atrium, and a tube was inserted to provide a fluid drainage path for blood and perfusate. To inhibit blood coagulation, heparin sodium (1500 units) was injected into the blood stream via hypodermic injection through the left ventricle and allowed to circulate for 2 minutes. An incision was then made in the left ventricle of the heart. A tube through which the fixatives were delivered was fed through the left ventricle into the aorta. The fixatives were pumped into the circulatory system via a peristaltic pump. First, 1 liter of warm (body temperature) saline was delivered as a pre-wash. Second, 1 liter of warm (body temperature) 1% paraformaldehyde in 25 mM cacodylate

buffer was delivered. Third, 1 liter of warm 3.5% glutaraldehyde in 25 mM cacodylate buffer was delivered. Finally, a second liter of 3.5% glutaraldehyde was administered, cold. Following perfusion fixation, the sciatic nerves and surrounding tissue were dissected from both legs. The excised tissue samples were further fixed in situ for 24 - 48 hours by immersion in cold glutaraldehyde to ensure stabilization at natural physiological length. After the 24 - 48 hours in glutaraldehyde, each sample was transferred to a cacodylate buffer solution for long-term storage.

Results

In all 4 animals, no difficulties were encountered during the implantation of the electrode around each nerve. In each case, the electrode appeared to fit easily and snugly, without requiring excessive movement or disruption of the nerve trunk. In one case, cat # 205, the animal experienced some oozing and bleeding from the surgical site on the right leg immediately post-operatively. This animal was administered additional sedatives so that the wounds could be cleaned and bandaged and bleeding could be stopped. Hematomas were observed in that leg for the following few days, but no other surgical complications were encountered.

All animals were found to be using and moving both hind limbs during the 10 day incubation period. In only the right (control) leg of one animal, cat #205, was slight favoring of the opposite leg displayed. This animal was still very active with no apparent loss of motion or strength in that limb. Favoring of the leg was most severe in the first post-operative days, appeared to be resolving, and likely was related to the hematoma described in the paragraph above.

In 3 of the animals, behavior, gait pattern, presence or absence of hock drop, presence or absence of knuckling of rear paws, and the animal's general attitude and activity level were observed over the course of the implant period. The basic neurological examination, comprised of checking paw withdrawal to light pinching, and toe-tapping during gait to observe limb corrections (placement reactions), was performed multiple times in these 3 animals over the 10 day period. Based on the behavior observations and neurological examinations, the animals appeared normal and were very active. The fourth animal was not handled due to its consistently poor attitude both before and after the surgical procedure which persisted throughout the duration of the experiment.

The aortic perfusions proceeded without incident. Upon explant, nerve cuffs in 2 of the animals were found subcutaneously at the site of the initial incision. How and why these cuffs did not stay on the nerve has not been determined. The cuffs, including those that remained in place and those that displaced from the nerve, and the lead wires, were found to be enclosed in a thin layer of encapsulation tissue.

Future Work

The excised tissue is being sectioned and prepared for histological processing. Once processed, the tissue will be evaluated for any neuronal changes or other signs of insult due to the surgical trauma or due to the presence of the cuff and lead.